A STUDY OF THE EFFECTS OF RECURRENT FREEZES ON WINTER WHEAT PLANTS

by

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TABLE OF CONTENTS

INTRODUCTION

In the wheat producing areas of the world, farmers lose many thousands of dollars each year due to abandoned acreage of wheat or reduced yields caused by winter killing or injury to the wheat plants. These large economic losses have stimulated a considerable amount of research to determine the cause of winter injury, factors influential in retarding it, methods for its prevention, and to develop or discover plants that are inherently resistant to the effects of such temperatures.

According to Salmon (22), the primary causes of winter killing are heaving, physiological drought, smothering and the freezing of the plant tissue. It now seems evident that by far the most destructive of these is the actual freezing of the plant. Freezing of the plant tissues may or may not cause death of the plant, depending upon such factors as the kind of plant, the kind of tissue, previous exposure to low temperature, and the intensity and duration of the cold.

The Kansas wheat crop is subjected each year to freezes throughout the winter season. During certain years these freezes have occurred after the wheat plants started their spring growth. As explained above, the causes of winter killing have been fairly well determined, but much remains to be learned as to the effect of sudden low temperatures on plants which have already withstood one or several previous freezes. At this time very little research of this nature has been carried on. It would seem advantageous to both the research man and the farmer to have a better understanding of the effects of repeated freezes on the wheat plants.

The results obtained in these tests involving recurrent freezes and their effect on the survival of winter wheat were obtained by use of the artificial refrigeration method of study and are contained and discussed herein.

REVIEW OF LITERATURE

The review of literature in this paper pertaining to low temperature relationships is divided into sections in order to present a clear picture of the possible causes and effects of low temperatures and other factors on winter survival. Previous work is reviewed under the following headings: (a) causes of winter killing, (b) hardening, and (c) measures of hardiness. There is also a section on the use of artificial refrigeration.

Causes of Winter Killing

The cause of injury by freezing, according to Miller (16), has been assumed to be due primarily to the withdrawal of water from the cell. Upon exposure to cold, water is usually withdrawn from the cell and freezes in the intercellular spaces. As the temperature falls, more water is frozen on the outer walls and more is drawn from the cell by imbibition.

According to Wiegand (29), the expansion of water in the cell is not sufficient to rupture the cell wall. He states, as originally noted by Muller-Thurgau in 1880, that ice forms within the cells only on very rapid freezing. He believes that death is due to water withdrawal from the cells to form ice, and not to the cold as such. In the opinion of Maximov (14), however, there is a mechanical deformation of the protoplasm as a result of being compressed by ice.

Doxter (4) concluded from work with wheat crowns that the withdrawal of water by ice formation was not a fully reversible process, but that in hardier plants it was more nearly so. Thus, when the plants are frozen beyond recovery, the water that has been removed by ice formation is not reabsorbed sufficiently to give the previous condition of turgidity.

Chandler (3), Abbe(1), and Wiegand (29) agree in that the injury is caused by rapid evaporation rather than rapid thawing. However, Akerman (2) found that death occurs when the plant is frozen, and not when it is thawed.

Several authors list some of the chemical-physical effects of water withdrawal upon the protoplasm. Newton (17, 18) states that precipitation of the proteins by the increasing salt concentration is a probable result of desiccation. Martin (15) believes the plant is killed because low temperatures coagulate and dehydrate the protoplasm to such an extent that it cannot take up water.

According to Miller (16), Muller-Thurgau took the view that death from low temperatures is due to a denaturing of the protoplasm that is caused by the exosmosis of water to the intercellular spaces where it forms ice masses.

Joslyn and Marsh (8) stated that the physical changes which occur during freezing depend chiefly upon ice formation and the changes are those concerned with the hydrolysis of pectins and sucrose. Other changes are in color and flavor.

Salmon (22) considers that desiccation of the protoplasm, mechanical injury caused by ice, chemical changes such as precipitation of the proteins and suspension of metabolism are all direct effects of low temperatures.

The recent view seems to be that frost killing consists in the withdrawal of water from the cells to form ice in the intercellular spaces, followed by the precipitation of the proteins in the protoplasm, due chiefly to the increased concentration of the cell sap.

Hardening

Previous investigators have shown that even rather sensitive plants can withstand relatively low temperatures if first subjected to a period of hardening. Several methods of hardening have been tried as measured by various physical and chemical tests.

Chandler (3) has published data which showed that slow wilting or partial withholding of water over a long period of time increased resistance to cold. Salmon and Fleming (23) and Harvey (5) demonstrated that plants are less succulent and consequently harden more when grown in a soil of low moisture content. Newton and Brown (19) observed a greater reduction of moisture content in hardy varieties due to the process of hardening.

Chandler (3) found that resistance to frost is increased by previous exposure to low temperatures. Suneson and Peltier (27) stated that a long exposure to medium low temperatures is necessary to build up maximum hardiness. They observed that the temperatures necessary to secure marked injury gradually had to be decreased as the season progressed. In November and early December they found seasonly high daily temperatures in conjunction with high radiation (conditions giving a maximum accumulation of organic reserves) most conducive to hardening of winter wheat. Subsequent exposure to sustained low temperature resulted in a further progressive increase in hardiness for about 3 weeks, at which time the maximum hardening for the season was reached. Akerman (2) discovered that wheat plants are more resistant during the coldest months, but become less so as the temperatures rise in the spring. Steinmetz (25) arrived at the same conclusion with alfalfa plants. Harvey (5) carried on extensive investigations with cabbage in relation to hardening. According to him, the growth rate is decreased so that the plants are smaller and more mature, and the leaves are considerably thicker. He watered plants with a solution of sodium chloride and found that growth was retarded and cold resistance increased. He also noted that protein was less easily precipitated from the juice of hardened plants.

Newton (18), working with winter wheat, observed no relation between the volume of press juice or the imbibition pressure of fresh leaves and resistance to cold with unhardened plants. However, there was a definite association when the plants previously had been exposed to hardening conditions. He also noted an increase in the quantity of sugar and hydrophylic colloids in hardened tissue.

Rosa (21), experimenting with certain vegetables, found that hardened plants retained more unfrozen water and had a greater total pentosan content. His work showed that hardened plants lose less moisture per unit of leaf area because of a lower transpiration rate.

Martin (13) sums up the effects of hardening. He states that "during the hardening of wheat there is a decrease in moisture content and an increase in total solids in the sap, freezing point depression of the sap, and imbibition pressure of the cell colloids."

Rosa (21) concluded that cold resistance will be increased by any form of treatment which materially checks growth.

Salmon (21) states that at greenhouse temperatures, winter hardened grain plants lose a perceptible amount of hardiness in 24 hours, and most of it in 120 hours. Laude (9) found that after 2 weeks the plants could withstand very little more freezing than if they had been grown continuously in the greenhouse, indicating that nearly all of the resistance to cold acquired outdoors had been lost. Working with previously hardened plants, Laude (10) concluded that the rate at which cold resistance is lost in varieties of winter wheat in the winter-spring transition is not necessarily associated with the degree of cold resistance in the hardened condition.

Measures of Winter Hardiness

Akerman (2) has been a leading exponent of the determination of sugar content as a criterion of cold resistance. He found an association between winter hardiness and sugar content in hybrid seggregates. Steinmetz (25) noted that a hardy variety of alfalfa contains more sugar, expressed in terms of total carbohydrates. According to Maximov (14), Lidforss was the first to publish work in regard to the protective role of soluble carbohydrates. Maximov considers that the protective action is physico-chemical, based on the concentration of the sugars. This increases the power of the cells to retain unfrozen water, and thus decreases the formation of ice. Mayer (15) and Newton and Brown (19), believe that the protective action which sugars exert against the precipitation of proteins is important. Newton (17) suggests that greater dormancy and a slower respiration rate may account for the lower rate of sugar loss from the leaves of hardy varieties in the late fall as compared to less hardy varieties.

Steinmetz (25) could find no correlation between dry matter in the leaves and resistance to cold. Akerman (2) found a close relationship between dry matter content and hardiness only with wide differences in hardiness. Martin (13) considers that hardy wheats are characterized by a high percent of total solids in the juice.

Newton (17, 18) found that the imbibition pressure of fresh leaves in the hardened condition generally was related directly to hardiness. He also showed that the volume of press juice of hardened leaves is inversely proportional to hardiness. According to the results of Tysdal (23), however, hardiness cannot be reliably measured by determining the quantity of press juice.

The quantity of hydrophylic colloids in the press juice of hardened tissues, as measured by the bound water, is directly proportional to hardiness as determined by Newton (17, 18) and

Newton and Brown (19).

Tysdal (28) used the viscosity of the cell sap as a measure of hardiness. He obtained a correlation of .9000 plus or minus .0386 between known rank in cold hardiness and viscosity and considers this property to be a consistent index of hardiness.

Harvey (6) has published a very extensive and complete bibliography of the low temperature relations of plants, including annotated references to 3,412 publications in this field. Levitt (11) has compiled a critical review regarding frost killing and hardiness of plants. For a further study of low temperature relations of plants, the reader is referred to these sources of information.

Use of Artificial Refrigeration

The use of artificial refrigeration as a measure of cold resistance has become increasingly important. Almost without exception a high correlation has been obtained between results of this method and field data. Harvey (5) was perhaps the first of the recent investigators to see the possibilities in, and to make use of, artificial refrigeration. He was closely followed by Akerman, in Sweden. Since this beginning, many investigators have found artificial freezing to be the best laboratory method for measuring the cold resistance of wheat. Akerman (2), Harvey (5), Laude (9, 10), Martin (13), Salmon (24), and Suneson and Peltier (27) have used this method successfully. These results indicate that artificial refrigeration is the most satisfactory test yet devised as a substitute for field data on winter hardiness.

MATERIALS AND METHODS

Pawnee wheat was planted in 4 inch pots on October 9, 1951 at the rate of 6 seeds per pot. Approximately 3000 pots were used. Pots used in the experiment averaged 5 plants each. The plants were grown outside in order to attain winter hardiness under as near natural field conditions as possible. Table 1 shows the daily maximum and minimum temperatures at Manhattan, Kansas for dates during which all or a portion of the plants remained outside. At the time artificial freezing operations began the plants were in good condition and had become sufficiently winter hardened to make the experiment feasible.

Artificial freezing operations began December 26, 1951 and continued until February 23, 1952. Plants were frozen in a freezing chamber, the walls of which were lined with a thick layer of cork. The low temperature was supplied by a direct expansion refrigeration plant. The refrigeration plant was thermostatically controlled, so that temperatures could be regulated as desired. An electric fan placed above the pots and near the center of the freezing chamber was effective in promoting uniform temperatures throughout.

A uniform freezing period of 23 hours, beginning at three o'clock was used throughout the experiment. This period was adopted, because considerable time is required for soil temperatures of the pots to approach that of the air in the freezing chamber.

Fifty pots taken from the bed of pots outside the greenhouse constituted each freezing group. Those pots of plants used in the first freezing trial (first freezing date) were designated as freezing group "A". Plants in these pots were frozen for 23 hours at the desired temperature and removed from the freezing chamber. Ten of the pots were placed in the greenhouse for observation, since it was assumed that plants kept in an atmosphere more favorable for growth would show effects of the low temperatures more quickly than if they had been placed outside. The remaining 40 pots were placed outside once more to recover from the effect of the low temperature under natural conditions. One week later plants in these pots were again frozen and 10 pots placed in the greenhouse for observation while the remaining 30 were returned to the outside bed. At the time of the second freeze, an additional group of 50 pots (freezing group "B") was frozen for the first time, so that 2 freezing groups were included; i.e., freezing group "B" was frozen for the first time while freezing group "A" was frozen for the second time. Ten pots of freezing group "B" were placed in the greenhouse and the remaining 40 pots again returned outside. One week after the second freezing trial, plants in the 30 pots remaining from freezing group "A", plants in the 40 pots remaining from freezing group "B", and 50 additional pots (freezing group "C") were placed in the freezing chamber. Similarily, freezing groups "D" and "E" were brought into the schedule at successive weekly intervals. By using this procedure, the wheat frozen on a single date included plants that were frozen for the first, second, third, fourth, and fifth times. In all cases 10 pots of each freezing group were transferred to the greenhouse for observation and the remainder placed outside to recover under natural conditions for one week. As the pots in each freezing group were exhausted, a new freezing group was brought into use. Under this system 5 different groups were being used at each freezing date. An attempt was made to obtain a representative sample from the outside bed of pots on the basis of condition and amount of top growth and also on numbers of plants per pot. After the freezing trial, the 10 pot sample to be placed in the greenhouse for observation was chosen in such a manner as to represent fairly the condition of top growth.

The relative effect of recurrent freezes on the wheat plant was measured in 2 ways: by estimating the percent of tissue above ground (top growth) that apparently was killed and by determining the percent of plants killed. The percent of top growth injury was estimated at 4, 8, and 12 days after freezing while the percent of plants killed was determined 8, 16, and 24 days after freezing. It was not feasible, if even possible, to measure exactly the percent of top growth injury and, therefore, the results reported are estimates of the amount of injury. Several estimates made independently by the author and major instructor agreed so closely, however, that this method was considered reliable for detecting differences in extent of damage.

Since it was desired to note the effect of recurrent freezes as determined by leaf injury as well as by mortality, both sets of data are given. The data presented give the percent injury

Table 1. Daily maximum and minimum temperatures for Manhattan, Kansas, October 9, 1951 to February 23, 1952.

Date	October		November		December		January		February	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1			37	27	64	36	37	7	56	26
2			37	15	54	41	17	6	57	18
3			34	17	68	44	29	7	58	23
4			55	17	56	24	40	9	44	24
5			29	19	50	30	35	8	52	25
6			29	19	55	39	26	1	45	18
7			39	21	54	28	30	4	49	20
8			42	24	48	25	43	23	65	27
9	66	40	58	32	32	11	44	22	45	20
10	74	44	65	28	37	15	39	9	66	25
11	76	49	70	32	41	21	42	9	65	22
12	80	53	60	44	47	27	48	24	66	24
13	78	56	71	45	52	25	49	25	47	39
14	75	47	61	30	29	14	59	26	45	34
15	76	44	57	30	18	-6	64	22	37	26
16	80	47	41	23	9	-6	64	22	37	26
17	71	43	21	19	9	-7	53	32	37	19
18	49	42	29	11	28	3	49	23	51	22
19	51	36	42	16	22	4	60	28	69	33
20	52	40	54	25	44	9	52	20	47	17
21	76	50	57	34	9	-6	41	22	39	12
22	73	34	62	29	20	-4	62	8	34	17
23	53	39	51	17	43	12	20	3	35	29
24	54	31	38	19	25	9	22	4		
25	68	37	31	20	28	11	37	13		
26	61	50	40	25	28	9	51	28		
27	58	34	49	22	25	5	43	20		
28	40	31	55	26	39	8	15	20		
29	66	35	62	29	48	26	38	7		
30	56	41	63	32	54	24	33	8		
31	53	30			64	25	55	17		

observed at the date of the last reading; i.e., the twelfth day after freezing for top growth injury and the twenty fourth day after freezing for plants killed.

In another part of the experiment crowns of frozen and unfrozen plants were examined. Each crown was cut longitudinally through its center. Care was taken to cut the crown in a manner so as to show a portion of the stem and the root system arising from the basal section of the crown as well as the crown tissue itself. The extent of injury to the interior crown tissue was compared with that of the top growth and with the percent of plants killed in the particular freezing group from which the crown was taken and with the ability of the plant to reestablish itself after the crown had been damaged.

EXPERIMENTAL RESULTS

Effect of Repeated Freezes at 4 Degrees Fahrenheit

Pawnee wheat previously hardened under natural outdoor conditions was artificially frozen from 1 to 5 times at 4 degrees Fahrenheit. Results of these successive freezes are given and discussed in this section of the report.

Data in Table 2 give the percent top growth killed as a result of subjecting plants to the successive freezes described above.

The percent of plants killed in those same experiments is given in Table 3. The top growth injury data in Table 2 and corresponding mortality data in Table 3 in each case were taken from the same group of plants.

Observations were made on samples of 10 pots, each sample being divided into 2 random groups of 5 pots each. These 5 pot samples were designated as "[illegible]" and "[illegible]". At the time of each freeze the samples for observation were placed in the greenhouse where conditions were favorable for recovery and growth. Estimates of injury to top growth and determinations of plant mortality were made on these samples. The condition of these plants was taken as an index of the entire lot of pots frozen on that particular date. All of the pots frozen, except the 10 that were placed in the greenhouse, were returned to the yard. At a later date these were frozen again and another 10 pot sample was chosen from among them and placed in the greenhouse for further readings. Thus, all figures in Tables 2 and 3 are based on the total accumulated amount of top growth injury and plant mortality resulting from the subjection of plants to 1 to 5 successive freezings. For example, in Table 2, freezing trial number one, plants which had not been artificially frozen previously had suffered 5.0 percent loss of top growth due to natural outside conditions. After being frozen one time at 4 degrees Fahrenheit, 10.0 percent of the top growth was dead, an increase of 5.0 percent as a result of the artificial freeze. Plants frozen for the first time in freezing trial number one were frozen the second time in freezing trial number 11. After being frozen artificially frozen 2 times, 40.0 percent of the top growth was dead, an increase of 30.0 percent over the 10.0 percent resulting from outdoor conditions and one artificial freeze. The plants received their third freeze in freezing trial number 21. At the end of this freeze a total of

Table 2. Percent top growth killed as a result of subjecting wheat plants to one or more freezes at 4 degrees Fahrenheit.

Freezing trial number	Number of artificial freezes											
	0		1		2		3		4		5	
	E*	W*	E	W	E	W	E	W	E	W	E	W
1	5	5	10	10								
2	5	5	20	20								
3	5	5	25	35								
4	5	5	50	50								
5	5	5	20	20								
11	5	5	40	40	40	40						
12	5	5	30	30	35	35						
13	5	5	40	40	40	35						
14	5	5	30	30	75	80						
15	5	5	35	35	45	40						
21	5	5	30	30	40	45	45	40				
22	5	5	25	25	45	50	40	50				
23	5	5	40	40	50	45	90	90				
24	5	5	35	35	80	80	95	95				
25	10	10	30	30	80	70	80	90				
31	5	5	40	40	70	70	80	80	90	90		
32	5	5	70	70	70	70	90	90	90	90		
33	10	10	40	40	80	80	80	90	95	95		
34	10	10	50	40	80	80	95	95	100	100		
35	10	10	30	30	60	60	90	90	100	100		
41	10	10	30	30	50	50	80	90	90	90	100	100
42	10	10	30	30	80	70	90	90	95	95	100	100
43	5	5	30	30	60	70	90	90	95	95	100	100
44	5	5	30	20	50	50	100	100	100	100	100	100
45	5	5	30	30	40	40	80	90	100	100	100	100
51	10	10	30	30	80	80	90	90	100	100	100	100
52	10	10	40	40	80	80	100	100	100	100	100	100
53	10	10	30	30	60	60	90	90	100	100	100	100
54	10	10	40	40	50	50	80	80	100	100	100	100
55	10	10	30	30	60	60	90	90	95	95	100	100

* Each freezing group used for observation was divided into 2 separate groups or samples of 5 pots each. "E" and "W" are used to designate these groups.

Table III. Percent plants killed as a result of subjecting wheat plants to one or more freezes at 4 degrees Fahrenheit.

Freezing trial number	Number of artificial freezes											
	0		1		2		3		4		5	
	E*	W*	E	W	E	W	E	W	E	W	E	W
1	0	0	0	4								
2	0	0	4	0								
3	0	0	8	19								
4	0	0	36	25								
5	0	0	0	0								
11	0	0	4	15	12	8						
12	0	0	10	20	12	12						
13	0	0	9	9	17	10						
14	0	0	11	11	44	48						
15	0	0	26	19	28	13						
21	0	0	4	7	24	24	25	23				
22	0	0	4	8	42	23	23	25				
23	0	0	28	14	19	23	81	76				
24	0	0	23	9	80	82	91	95				
25	0	0	11	28	85	62	86	92				
31	0	0	30	31	61	77	96	91	93	92		
32	0	0	77	79	96	98	96	96	77	87		
33	5	8	52	62	85	72	72	85	100	100		
34	0	0	16	23	100	100	100	100	100	100		
35	0	0	30	33	94	85	97	89	100	100		
41	4	0	40	33	70	89	90	89	100	100	100	100
42	5	4	18	19	100	85	100	100	100	100	100	100
43	0	0	15	27	61	73	89	85	100	96	100	100
44	0	0	22	18	42	65	100	100	100	100	100	100
45	0	0	13	20	62	46	100	100	100	96	100	100
51	0	0	9	8	42	44	92	92	100	100	100	100
52	0	0	8	8	48	69	100	100	100	100	100	100
53	0	0	4	18	50	61	78	68	100	96	100	100
54	0	0	8	33	42	30	62	61	100	100	100	100
55	0	0	8	15	30	40	69	78	100	100	100	100

* Each freezing group used for observation was divided into 2 separate groups or samples of 5 pots each. "E" and "W" are used to designate these groups.

45.0 percent and 40.0 percent reduction in top growth was estimated in "E" and "W", respectively. At the end of the fourth artificial freeze, in freezing trial number 31, 90.0 percent of the top growth had been killed, an increase of 45.0 percent and 50.0 percent (as estimated in "E" and "W") due entirely to this particular freeze. Finally, the plants were artificially frozen for the fifth time in freezing trial number 51. No live top growth was noted after this freeze and it was assumed that 100 percent of the top growth was dead.

In each case, the same plants frozen artificially in any specific freezing trial were again frozen 5 freezing trials later. Thus, plants frozen for the first time in freezing trial number 5 were frozen for the second, third, fourth, and fifth times in freezing trial numbers 15, 25, 35, 45, and 55, respectively.

The method of recording the data followed in Table 2 was also used in Table 3. As mentioned previously, the top growth injury data in Table 2 and the corresponding mortality data in Table 3 were taken from the same group of plants. For example, plants which received their second artificial freezing in freezing trial number 12 suffered a loss of 30.0 percent top growth (shown in Table 2) and an average of 12.0 percent mortality (shown in Table 3).

The differences between paired values of "E" and "W" represent sampling variation and served for the computation of error variance. It appeared evident that error variance computed on all of the available data (n = 130) would be a better estimate of experimental error than if computed on a portion of the experiments. In the statistical analysis, therefore, the error variance was computed as a separate problem, using the formulae

$$\text{Sum of Squares} = \text{Sum of } Y^2 - \frac{(\text{Sum of } Y)^2}{n} \quad \text{and}$$

$$\text{Variance} = \frac{\text{Sum of Squares}}{\text{Degrees of Freedom}}$$

as outlined by Paterson (20). Error variance for both top growth injury and plant mortality were computed and used in testing the interaction and main effects for statistical significance. Results of these computations are given in Tables 5 and 6.

The average percent of top growth and plants killed by freezes at 4 degrees Fahrenheit and the percent of damage to top growth and plants remaining after the preceding freeze are shown in Table 4. It appears that a reduction in top growth of approximately 1/3 was sufficient to cause death of almost 1/5 of the plants. The percent top growth killed and the percent of plants killed increased with each freeze. It is interesting to note the close relationship between these 2 factors as the number of freezes was increased. After 2 artificial freezes plants showed almost the same degree of top growth injury and mortality. Only 2 freezes at 4 degrees at 4 degrees were required to kill almost 60.0 percent of top growth and cause the death of more than 1/2 the plants. As is shown by the data in Table 4, only 2 or 3 successive freezes at 4 degrees were necessary to so materially reduce plant growth and plant numbers that the plants were almost or entirely worthless from the practical and financial standpoint of the wheat producer. If field conditions were closely related to conditions of this experiment, farmers would

Table 4. Average percent of top growth and plants killed by freezes at 4 degrees Fahrenheit, the amount of top growth injury and mortality added by each freeze, and the percent of damage to top growth and plants remaining after the preceding freeze.

Number times frozen	Top growth killed: Accumulated percent	Top growth killed: Percent added by each freeze	Plants killed: Accumulated percent	Plants killed: Percent added by each freeze	Percent damage to top growth and plants remaining after the preceding freeze: Injury	Percent damage to top growth and plants remaining after the preceding freeze: Mortality
1	33.5	26.7*	19.0	18.5*	28.6	18.6
2	59.8	26.3	52.6	33.6	43.9	41.5
3	83.9	24.1	82.0	29.4	60.1	62.0
4	96.7	12.8	97.9	15.9	79.5	88.3
5	100.0	3.3	100.0	2.1	100.0	100.0

* 6.8 percent of top growth and 0.5 percent of plants were killed by natural outside temperatures.

surely abandon the acreage devoted to wheat.

It must be emphasized at this point, however, that the results discussed herein are not those obtained under natural field conditions where a snow cover and natural soil insulation tend to reduce injury appreciably. However, it seems reasonable to assume that results of successive freezes under experimental conditions give an indication of trends of top growth injury and mortality which would occur due to several successive freezes under natural field conditions of the farm.

A highly significant correlation of .958 at 59 degrees of freedom was found between percent top growth injury and percent plants killed, showing that a very definite association did exist (see Table 7). From data presented and results of a statistical analysis, then, a reduction of more than 1/4 of the top growth would be expected, in the majority of instances, to result in an increase of plant mortality.

The effect of repeated or recurrent freezes on winter wheat may be determined by comparing the effects of each successive freeze on the plants that survived the preceding freeze. Data of this nature are shown in Table 4. Since plants were frozen for the first, second, third, fourth, and fifth times on the same date; i.e., were in the freezer at the same time, it is possible to directly compare these data.

As shown in Table 4, natural weather conditions accounted for the injury of 6.8 percent of top growth and the killing of 0.5 percent of the plants before they were artificially frozen. Of the material remaining, the first artificial freeze at 4 degrees

Table 5. Analysis of variance of top growth injury in freezing trial numbers 41-45 and 51-55 resulting from freezes at 4 degrees Fahrenheit.

	df	ss	var.	F
Between freezing trials	9	859.09	95.45	2.14*
Between number of times frozen	5	71387.42	14277.48	319.91**
Interaction: freezing trials x number of times frozen	45	2008.41	44.63	6.59**
Total	59	74254.92	1241.61	
Error variance[1]	129	873.27	6.77	

* Significant at 5 percent level
** Significant at 1 percent level
1 Error variance was computed as a separate problem and used in testing the interaction and main effects for statistical significance.

Table 6. Analysis of variance of mortality in freezing trial numbers 41-45 and 51-55 resulting from freezes at 4 degrees Fahrenheit.

	df	ss	var.	F.
Between freezing trials	9	1570.65	174.52	2.10
Between number of times frozen	5	92649.77	18529.95	222.89**
Interaction: freezing trials x number of times frozen	45	3741.17	83.14	2.17**
Total	59	97961.59	1660.36	
Error variance[1]	129	4932.96	38.24	

** Significant at 1 percent level
1 Error variance was computed as a separate problem and used in testing the interaction and main effects for statistical significance.

Table 7. Analysis of co-variance showing correlation of top growth injury and plant mortality for freezing trial numbers 41-45 and 51-55 resulting from freezes at 4 degrees Fahrenheit.

Factors	df	ss_x	ss_y	sp_{xy}	r
Between freezing trials	9	859.09	1570.65	907.82	.773
Bet. no. of times frozen	5	71387.42	92649.77	81105.95	.997**
Int: freezing trials x no. of times frozen	45	2008.41	3741.17	1940.38	.706
Total	59	74254.90	97961.59	81678.60	.958**

** Significant at one percent level

injured 28.8 percent of the top growth and killed 18.6 percent of the plants. This evidence suggests that plants can withstand a substantial amount of injury before they are killed by freezing.

The initial 4 degree freeze seemed to weaken the surviving plants, since they were more severely injured and a higher percent of the plants were killed by the second 4 degree freeze than by the first. Data reported in Table 4 show that the second freeze killed 43.9 percent of the top growth and 41.5 percent of the plants that survived the first 4 degree freeze. These figures also indicate that the plants surviving the initial 4 degree freeze were in a somewhat weakened condition in that a level of top growth injury resulted in almost the same level of mortality. This would seem to indicate that the top growth could not absorb further damage before the plant began to die.

The hypothesis that plants are weakened by a freeze so that the surviving plants are less able to withstand a subsequent freeze seems to be substantiated also by results of the third, fourth, and fifth successive freezes. The third freeze injured 60.1 percent of the top growth and killed 62.0 percent of the plants remaining after the second freeze as compared to 43.9 percent top growth injury and 41.5 percent mortality of plants surviving the second freeze at 4 degrees. The data also show that freezing of plants surviving 2 previous 4 degree freezes resulted in about the same percent of injury as mortality.

The fourth successive 4 degree freeze resulted in the injury of 79.5 percent of the top growth and the death of 88.3 percent of the plants surviving the third freeze. Again, there was a higher percent of top growth injury and mortality among plants surviving a freeze than among the survivors of a preceding freeze.

After 4 successive freezes at 4 degrees only 3.3 percent of the top growth was estimated to be alive, while 2.1 percent of the plants had survived. A subsequent freeze at 4 degrees completely killed the remaining top growth and plants.

From an examination of the data reported in Table 4, it would seem that plants previously not frozen artificially were able to withstand at least some injury before mortality became severe, but that a freeze at 4 degrees weakened the top growth and plants to the extent that a subsequent freeze at the same temperature resulted in about equal percent mortality and top growth injury among the survivors of the first freeze.

Each successive freeze resulted in a higher degree of injury and greater mortality of the plants that survived the preceding freeze. The results of these experiments, then, indicate clearly that plants are weakened more and more by each successive freeze and, consequently, are less able to withstand subsequent freezes.

Figure 1 presents graphically data given in Table 4 and tends to show more clearly the association.

The plants in Plate I give an indication of the amount of recovery made in the greenhouse by plants frozen from 1 to 5 times at a temperature of 4 degrees Fahrenheit. The photographs also indicate the numbers of plants surviving such freezes. Plants which were not frozen in the freezing chamber (Pot 1) suffered approximately 6.3 percent loss of top growth and averaged only 0.5 percent mortality. This damage was due to natural outside temperatures. Pot 2 of Plate I illustrates the condition of plants artificially frozen once, Pot 3 those frozen twice, etc. As can be seen from the plate, more top growth and plants were dead at the end of each freeze than at the end of the previous freeze. The condition of plants in Pots 3 and 4, which were frozen 2 and 3 times, respectively, points out the accumulative effect of recurrent freezes. After 4 freezes there remained only one live plant and it had lost much of its functional leaf surface. At the end of the fifth freeze all plants apparently were dead.

Approximately 2 weeks after the final reading, a few plants which had been classed as dead started showing life again. Examination showed that from 1 to 3 axillary buds on each of these plants had not been entirely killed and were growing. However, these never developed to any great extent and soon died. A possible explanation for the short duration of these axillary buds will be considered and discussed later in this report.

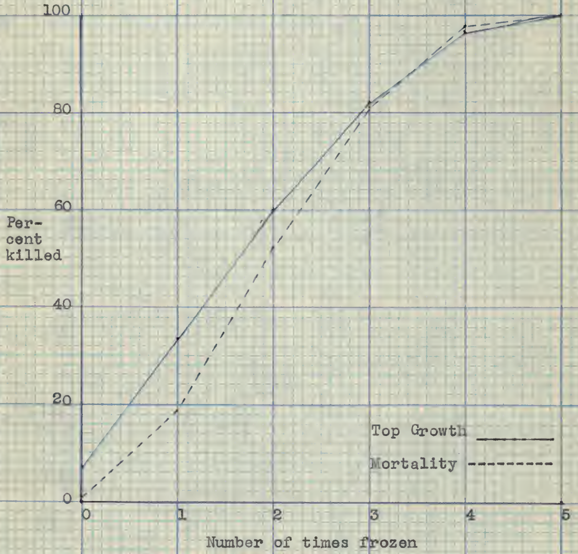

Fig. 1. Relation of percent top growth killed and percent mortality caused by one or more freezes at 4 degrees Fahrenheit.

EXPLANATION OF PLATE I

Plants in this photograph are representative samples of lots frozen from 1 to 5 times. After being frozen, the plants were placed in the greenhouse where conditions were more favorable for growth. The photograph was taken 3 weeks after the plants were frozen.

Pot 1. Plants which were not artificially frozen.

Pot 2. Plants which were frozen one time at 4° F.

Pot 3. Plants which were frozen 2 times at 4° F.

Pot 4. Plants which were frozen 3 times at 4° F.

Pot 5. Plants which were frozen 4 times at 4° F.

Pot 6. Result of freezing plants 5 times at 4° F.

PLATE I

A statistical analysis of the data showed that significantly more tissue was destroyed and more plants killed with each successive freeze, with the exception of the difference between freezes number 4 and 5 (least significant difference between freeze means equaled 3.47 for top growth and 6.25 for mortality). Each additional freeze, then, except the fifth resulted in a statistically significant increase in top growth and plants killed as a result of the freeze. In this instance, lack of living material seemed to be the limiting factor and resulted in the low amount of injury and mortality resulting from the fifth freeze, since 96.7 percent of top growth and 97.9 percent of all plants were killed as a result of the preceding 4 freezes.

Effect of Freezing Wheat at 4 Degrees Followed by Freezing at 10 Degrees

An attempt was made to determine the effect of a freeze at 10 degrees on plants which had not been frozen artificially and on plants which had suffered one or more previous artificial freezes. With this purpose in mind, plants were frozen once, twice, and three times at 4 degrees. A portion of each freezing group was refrozen at 10 degrees so that each group of plants frozen one or more times at 4 degrees was subjected to an additional freeze at 10 degrees. Results of this experiment are shown in Tables 8 and 9. The experiment was repeated 6 times. The data in Table 8 show that each time the plants were frozen there was a considerable increase in the amount of top growth killed. Likewise, additional plants were killed at each freezing, as is shown in Table 9.

Table 8. Percent of top growth killed as a result of subjecting winter wheat plants frozen previously at 4 degrees to a 10 degree freeze.

Number and temperature of freezes	Experiment number					
	61	62	63	64	65	66
No artificial freeze	10.0*	10.0*	10.0*	10.0*	10.0*	10.0*
1 freeze at 10 degrees	30.0	40.0	30.0	30.0	30.0	30.0
1 freeze at 4 degrees plus a 10 degree freeze	70.0	90.0	85.0	90.0	80.0	80.0
2 freezes at 4 degrees plus a 10 degree freeze	80.0	97.5	90.0	97.5	92.5	95.0
3 freezes at 4 degrees plus a 10 degree freeze	90.0	97.5	95.0	100.0	95.0	95.0

* Due to natural outside temperatures

Table 9. Percent of plants killed as a result of subjecting winter wheat plants frozen previously at 4 degrees to a 10 degree freeze.

Number and temperature of freezes	Experiment number					
	61	62	63	64	65	66
No artificial freeze	0	0	0	0	0	0
1 freeze at 10 degrees	42.0	25.4	27.2	20.9	20.0	16.5
1 freeze at 4 degrees plus a 10 degree freeze	72.1	83.6	66.0	88.0	54.0	81.8
2 freezes at 4 degrees plus a 10 degree freeze	86.0	90.2	82.2	96.0	88.0	96.1
3 freezes at 4 degrees plus a 10 degree freeze	95.5	98.1	96.0	100.0	94.8	98.0

As reported in Table 10, plants which had not been frozen previously by artificial means were injured to the extent of 21.7 percent additional top growth killed and 36.5 percent of plants killed when subjected to a single 10 degree freeze. This amount of damage was almost as much as that added by a 4 degree freeze when the plants were in a hardened condition as shown in Table 4. This greater damage may have been due to the loss of some winter hardiness with the advance of the season, as the experiments reported in Tables 8 and 9 were made from February 11 to February 23, while data reported in Tables 2 and 3 were made from December 26 to February 11. Just previous to the time the later experiments were begun weather conditions were fairly mild for several days, as is shown in Table 1. As was pointed out in the review of literature, wheat plants sometimes lose a great deal of winter hardiness in a very short time. Salmon (23) found that a greenhouse temperatures winter hardened grain plants lost a perceptible amount of hardiness in 24 hours and much of it in 120 hours. Laude (9) found that after 2 weeks at greenhouse temperatures nearly all cold resistance had been lost. During the 10 day period preceding these experiments the average daily temperature was 33.2 degrees Fahrenheit, with the average on 5 of these days above 40 degrees (see Table 1). While these temperatures were obviously not optimum greenhouse temperatures, it seems reasonable to assume they were sufficiently high for the plants to lose some hardiness, since plants have been observed to actually make some growth at a temperature of 40 degrees. Newton and Brown (19) suggest that cold hardiness is determined, in part, by the protective action of certain sugars and that dormancy

Table 10. Average percent top growth injury and mortality of plants resulting from and added by freezes at 4 degrees, at 10 degrees, and by both 4 and 10 degrees and the percent of damage to top growth and plants remaining after each of these freezes.

Number and temperature of freezes	Injury	Mortality	Percent added by additional freezing				Percent of damage to top growth and plants remaining after preceding freeze			
			At 4°		At 10°		At 4°		At 10°	
	%	%	Inj.	Mort.	Inj.	Mort.	Inj.	Mort.	Inj.	Mort.
No artificial freeze	10.0	0								
1 freeze at 10 degrees	31.7	35.5			21.7	35.5			24.1	35.5
1 freeze at 4 degrees	55.0	65.5	45.0	65.5			50.0	65.5		
1 freeze at 4 degrees plus a 10 degree freeze	82.5	74.4			27.5	8.9			61.1	25.8
2 freezes at 4 degrees	82.5	77.3	27.5	11.8			61.1	31.3		
2 freezes at 4 degrees plus a 10 degree freeze	92.1	89.8			9.6	12.5			54.0	55.0
3 freezes at 4 degrees	90.0	88.2	7.5	10.9			42.9	48.0		
3 freezes at 4 degrees plus a 10 degree freeze	95.4	97.1			5.4	8.9			54.0	75.4

and slow respiration rate may account for the low sugar loss of hardy varieties. They also suggest that as temperatures rise, the respiration rate is increased with the loss of sugar and cold hardiness the final result. They believe that the normal respiration of the living plant during the winter gradually decreases the nutrient reserves which probably lessens cold hardiness in late winter. Thus, it is quite probable that the average daily temperatures noted above were fully high for the rate of sugar loss to increase and the amount of cold hardiness to decrease by the time these experiments were begun. Also, Suneson and Peltier (26) found the highest amount of winter hardiness approximately 3 weeks after early December and that winter hardiness was gradually reduced after that time. Their findings would indicate that plants used in these experiments had lost some of their winter hardiness, since the experiments were begun some 42 days after the maximum hardiness point probably had been reached.

As was the case with plants frozen at 4 degrees, there was a highly significant correlation of .885 at 23 degrees of freedom between percent of top growth killed and percent of plants killed (see Table 13). The relationship between top growth injury and plant mortality is shown in graphic form in Fig. 2. Methods of sampling and computation of error variance were the same as outlined for experiments with successive 4 degree freezes. Results of the analysis of variance and co-variance are reported in Tables 11, 12, and 13.

Data reported in Table 10 show a comparison of the average percent injury to top growth and plants killed by a freeze at 4

Table 11. Analysis of variance of top growth injury resulting from freezes of 4 degrees and 10 degrees.

	df	ss	var.	F
Between freezing trials	5	38.55	7.71	.029
Between number of times frozen	3	15435.42	5145.13	19.27**
Interaction: freezing trials x number of freezes	15	4005.20	267.01	39.44**
Total	23	19479.17	846.92	
Error variance[1]	24	162.48	6.77	

** Significant to 1 percent level
1 Error variance was computed as a separate problem and applied to the results.

Table 12. Analysis of variance of mortality resulting from freezes of 4 degrees and 10 degrees.

	df	ss	var.	F
Between freezing trials	5	420.71	105.18	2.05
Between number of freezes	3	18753.66	6175.87	120.47**
Interaction: freezing trials x number of freezes	15	975.85	65.06	1.27
Total	23	20150.22	876.10	
Error variance[1]	24	1230.96	51.29	

** Significant to 1 percent level
1 Error variance was computed as a separate problem and applied to the results.

Table 13. Analysis of co-variance showing correlation of top growth injury and mortality resulting from freezes of 4 degrees and 10 degrees.

Factors	df	ss_x	ss_y	sp_{xy}	r
Bet. freezing trials	5	38.55	420.71	104.56	.821*
Bet. no. times frozen	3	15433.42	18753.66	17134.63	.995**
Int: freezing trials x no. times frozen	15	4005.20	975.83	1540.21	.782*
Total	23	19479.17	20150.22	17486.75	.885**

* Significant to 5 percent level
** Significant to 1 percent level

degrees and top growth and plants killed by a 10 degree freeze after one or more freezes at 4 degrees. Also shown in Table 10 is the percent of damage to top growth and plants remaining after the preceding freeze.

Plate II shows a comparison of recovery of wheat which had not been frozen artificially with wheat frozen once at 10 degrees and at 10 degrees after one previous 4 degree freeze. The photograph was taken 2 weeks after freezing. Plants in Fig. 1 of Plate II were estimated to have lost an average of 10.0 percent of their top growth as a result of exposure to outdoor winter conditions. There was relatively no mortality in these plants. Plants in Fig. 2 show the effect of a 10 degree freeze and plantsin Fig. 3 the effect of a 10 degree freeze after a 4 degree freeze.

Data reported in Table 10 are not consistent in showing whether plants damaged by cold are more or less susceptible to damage by subsequent exposure to cold temperatures. In these experiments damaged plants on the average suffered about as much additional damage when exposed to freezing at 10 degrees as at 4 degrees.

EXPLANATION OF PLATE II

Plants in this photograph are representative samples of lots not frozen artificially and of plants frozen at 4 degree and 10 degree temperatures. After being frozen, the plants were placed in the greenhouse where conditions were more favorable for growth. The photograph was taken 2 weeks after the plants were frozen.

Fig. 1. Plants which were not artificially frozen.

Fig. 2. Plants which were frozen one time at 10 degrees Fahrenheit.

Fig. 3. Plants which were frozen one time at 4 degrees Fahrenheit and again at 10 degrees Fahrenheit.

PLATE II

Fig. 1 Fig. 2 Fig. 3

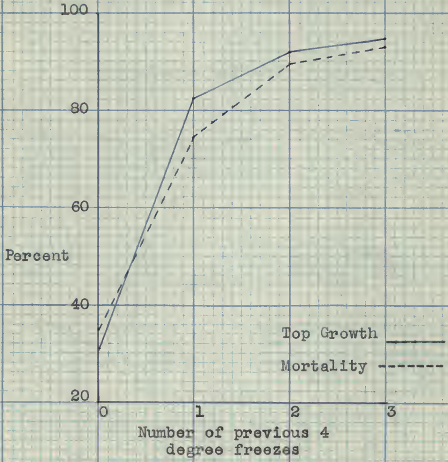

Fig. 2. Relation of percent top growth killed and percent mortality caused by a 10 degree freeze after previous 4 degree freezes.

In attempting to explain the differences in effects of the various combinations of temperatures to which plants were subjected, it must be remembered that by the time these experiments had begun the plants had lost a considerable amount of the hardiness they had gained previously. Thus, it is reasonable to assume that plants in this condition might be injured severely by a sudden exposure to a low temperature. This assumption appears to be true, since, as was shown in Table 10, a temperature of 10 degrees was sufficient to injure 1/5 of the top growth and kill about 1/3 of plants remaining from the effect of natural outdoor temperatures, while a freeze of 4 degrees injured 1/2 of the top growth and 2/3 of remaining plants were killed; i.e., the freeze at 4 degrees resulted in approximately twice the damage caused by the 10 degree freeze.

No information was obtained on plants frozen at 10 degrees after being frozen 4 or 5 times previously at 4 degrees, but it was assumed the same effects would be noted; i.e., essentially no difference in the effects on remaining plant material from freezes at 10 degrees and 4 degrees after one or more freezes at 4 degrees. In all probability, however, subsequent freezes at either 4 or 10 degrees would add less additional damage to plants as the number of freezes increased, since the upper limit must soon be reached where no plant material would be alive.

Effect of Freezing Wheat at 4 Degrees During "Winter" and "Spring" Stages

During certain years winter wheat in Kansas has been subjected to freezing temperatures at or just before the time the plants started spring growth. As noted previously, average daily

Table 14. Average amount of injury and plant mortality added by successive freezes of plants which were fully hardened as compared to those frozen in a semi-hardened condition and the percent of damage to top growth and plants remaining after each of these freezes.

Number of 4° freezes	Top growth injury		Mortality		Percent of damage to top growth and plants remaining after preceding freeze			
	Hardened winter condition	Semi-hardened early spring condition	Hardened winter condition	Semi-hardened early spring condition	Top growth injury		Mortality	
					"Winter"	"Spring"	"Winter"	"Spring"
1	26.7	45.0	18.5	65.5				
2	26.3	27.5	33.6	11.8	35.9	50.0	41.1	33.2
3	24.1	7.5	29.4	10.9	51.3	27.3	61.4	48.0
Total damage by 3 freezes	77.1	80.0	81.5	88.2				

temperatures were sufficiently high for the plants to have lost at least some of their previously acquired cold hardiness. Data reported in Table 14 give a comparison of damage to plants resulting from freezes at 4 degrees during the "winter" period when plants were fully hardened and during the "spring" period after plants had lost at least a portion of their winter hardiness.

A single freeze at 4 degrees resulted in almost twice the top growth injury and more than 2 1/2 times as much mortality after the plants had lost part of their winter hardiness than when frozen in a hardened condition. It is interesting to note that after a single freeze at 10 degrees when plants were in the "spring" condition, almost as much top growth was dead and almost twice the numbers of plants were dead as when the plants were frozen once at 4 degrees in their fully hardened condition (see Table 14).

Two freezes at 4 degrees seemed to add about the same amount of additional damage to top growth, but fewer additional plants were killed during the "spring" freezes, according to data reported in Table 14. Three freezes at 4 degrees resulted in less injury and mortality among plants surviving the second freeze in the "spring". At first sight it would appear that each successive freeze during the "spring" affected the plants less, and that perhaps those plants surviving the initial freeze were hardened to better withstand the subsequent freezes. However, this difference could be due to an ecological advantage, or to the fact that the upper limit of remaining plant material was being reached when plant losses would necessarily be lowered. Thus, the assumption that plants were hardened by the first freeze to better withstand subsequent freezes cannot be proved to a great degree of assurance.

Effect of Freezing on the Crown Tissue

It was evident that the number of plants which were apparently recovering 8 days after freezing was greater than at the end of 16 and 24 days. This was due to the injury of some plants which at first showed signs of life, but later died. In an effort to determine the type of injury which could possibly be the cause of further plant deaths, crowns of unfrozen and frozen plants were examined and compared.

Plate III shows the interior of crown tissue of plants frozen at a 4 degree temperature 1 to 5 times as compared to that of an unfrozen plant. The crown taken from an unfrozen plant (Fig. 1 of Plate III) shows unstained white tissue from the top of the stem extending down through the base of the crown. The roots are vigorous, well branched and undamaged. Figure 1 of Plate I gives an indication of the average growth of a plant of this nature. As reported in Table 4, only 6.8 percent of top growth and 0.5 percent of the plants were killed by natural conditions.

The crown of a plant frozen one time at 4 degrees is shown in Fig. 2 of Plate III. The tissue appears to be healthy in the upper part of the crown and stem, but in the lower part of the crown the tissue had turned dark in color. This dark tissue had been killed as a result of the freeze. On the average, as shown in Table 4, about 1/3 of the top growth and almost 1/5 of the plants were killed. The plant shown in this figure remained vigorous, although it had been injured.

The plant shown in Fig. 3 of Plate III had been frozen 2 times at 4 degrees. The interior of the crown shows a more definite darkening of the lower part extending upward into the lower portion of the center stem. Stems and roots of this plant appear to be in good condition, but there was a marked reduction in top growth, and, as reported in Table 4, more than 1/2 the plants were killed as a result of the 2 freezes.

The crown shown in Fig. 4 of Plate III was taken from a plant frozen 3 times at 4 degrees. This crown had dead tissue covering its entire base and extending upward to include the left stem. At the time the photograph was taken, the stem tissue was very wet. As a result, the dead tissue is not clearly defined as in most of the other cases. However, it appears to cut entirely across the crown separating a living root (center) from a living stem (right). As reported in Table 4, the reduction in top growth and numbers of plants amounted to about 80.0 percent after 3 freezes at 4 degrees.

A crown from a plant frozen 4 times at 4 degrees is shown in Fig. 5 of Plate III. The average percent of top growth killed of plants in this class was 90.7 percent while 97.9 percent of the plants were killed. It is easily seen that such would be the case, since the tissue of the crown as well as that of the left and center stems is dead.

The crown in Fig. 6 came from a plant frozen 5 times at 4 degrees. Both the crown and the right stem were dead, but showed a light color on the photograph for the same reasons as given for the appearance of the crown in Fig. 4. Plants in this class averaged 100 percent mortality and loss of live top growth.

From the data presented, it would seem that there is a definite association between the condition of the crown tissue and the percent live top growth remaining and plant survival. However, a small amount of injury to the crown tissue does not seem to depress regrowth after freezing or cause mortality to a great extent. As the injury to the crown increases, though, there seems to be a more definite increase in percent top growth injury and loss of plants.

Plate III shows that frost injury to crown tissue is at first confined to the basal portion of the crown, but extends upward as the injury increases. In the final stage all the crown tissue, the stems, and part of the roots have turned a dark brown or black in color indicating that there is no longer live tissue.

As pointed out previously, plants which were apparently recovering from injuried resulting from freezes suddenly die. The crown in Fig. 5 of Plate III may be used to good advantage in discussing the possible cause of this occurrence. The photograph of the crown shows that the upper portion of the left and center stems was still alive and apparently still functioning. The right stem seems to have had live tissue throughout. The general condition is borne out by the appearance of the corresponding plants in Fig. 5 of Plate I; i.e., a great reduction of top growth and at least one stem still functioning. Even though portions of the stem were still alive, however, it would be very questionable to assume that they would have continued to live for longer than a few days or a week, since all vascular connections to the roots had been severed due to the death of the entire crown tissue. Thus, it is

EXPLANATION OF PLATE III

Crowns in this photograph are representative samples of those taken from plants which were frozen from 1 to 5 times. The crowns are from plants which had been recovering in the greenhouse for 3 weeks, at which time the photograph was taken.

Fig. 1. A crown from a plant not artificially frozen.

Fig. 2. A crown from a plant which was frozen one time at 4° F.

Fig. 3. A crown from a plant which was frozen 2 times at 4° F.

Fig. 4. A crown from a plant which was frozen 3 times at 4° F.

Fig. 5. A crown from a plant which was frozen 4 times at 4° F.

Fig. 6. A crown from a plant which was frozen 5 times at 4° F.

PLATE III

Fig. 1 Fig. 2 Fig. 3 Fig. 4 Fig. 5 Fig. 6

possible for a wheat plant to appear very much alive and to apparently be recovering from a freeze, yet suddenly die when nutrients and water above the dead crown tissue have been exhausted.

By comparing crowns of Figs. 2 and 3 of Plate III with plants in Figs. 2 and 3 of Plate I it is evident that in crown tissue which has not been too severely injured there may yet be sufficient vascular connections between tops and roots for the plants to live. This hypothesis could be correct, even though, as was pointed out in the discussion of Table 4, top growth may be materially reduced and percent mortality greatly increased when the plants are compared to unfrozen plants or those which have been frozen only 1 or 2 times.

SUMMARY

Pawnee wheat was frozen at 4 degrees and at 4 and 10 degrees in a series of experiments at Manhattan, Kansas, beginning December 26, 1951 and ending February 23, 1952. Freezing equipment consisted of a freezing chamber surrounded by a thick layer of cork with a thermostatically controlled refrigeration plant used to provide the cold temperatures. The design of the experiment was such that plants could be frozen for the first, second, third, fourth, and fifth times on a single freezing date; that is, they were all in the freezer at the same time. Plants were allowed one week to recover outside the greenhouse before being frozen again. The relative effect of the recurrent freezes was measured by estimating the percent of top growth apparently killed and determining the percent of plants killed. In another part of the experiment crowns of frozen and unfrozen plants were examined and compared.

An increasing proportion of top growth injury and plant mortality resulted as the number of 4 degree freezes increased from 1 to 5. At the end of the fifth freeze all top growth and plants were dead. Each successive freeze seemed to weaken plants more and more so that they were less able to withstand subsequent freezes.

Plants previously not frozen artificially seemed to be able to withstand considerable injury to top growth before plants were killed. An initial freeze, however, seemed to weaken the plants, so that as the number of subsequent freezes was increased an increasingly greater proportion of the living tissue was killed.

To determine whether a plant was weakened by a 4 degree freeze so that a subsequent freeze at a higher temperature might cause more injury and higher mortality, an experiment was planned whereby plants were frozen 1, 2, and 3 times at 4 degrees. Each freezing group was then refrozen at 10 degrees and the results compared with the previous experiment in which a 4 degree temperature was used. The data proved to be very inconsistent, but in general it was found that when healthy plants previously unfrozen by artificial means were frozen, a 4 degree freeze caused considerably more damage than a freeze at 10 degrees, but essentially no difference was noted between these temperatures during subsequent freezes.

Plants frozen at 4 degrees while in a semi-hardened condition were injured and killed much more than when frozen at the same temperature while in a hardened condition. There appeared to be no great difference in amount of top growth injured and number of plants killed after 2 freezes at 4 degrees. Three freezes at 4 degrees appeared to result in less injury and mortality to plants in a "spring" condition. However, this difference may have been due to an ecological advantage, or the fact that the percent of injury and mortality was approaching a point above which no plants were alive, and, thus, cannot be said to definitely be caused by a hardier condition of the plants.

It was noted that the number of plants apparently surviving a given freeze was greater on the eighth day than on the sixteenth or twenty fourth day after freezing. To determine the cause of this occurrence, crowns of frozen and unfrozen plants were examined and compared. It was found that it was possible for plants to continue to function even though a portion of the crown tissue was dead, but that if injury to the crown tissue was too severe, plants might die as a result of the cutting off of water and nutrients rather than injury to the top growth itself.

CONCLUSIONS

From an examination of the data and personal observations several conclusions have been formulated as to the effect of recurrent freezes on winter wheat plants:

Under laboratory conditions, where no snow cover or other natural protection was present, 3 freezes at a temperature of 4 degrees resulted in injury so severe as to make the wheat plant no longer of economic value. As few as 5 recurrent freezes at this temperature under the above conditions resulted in 100 percent killing of the wheat plants.

An increasing proportion of top growth injury and plants killed may be expected as the number of successive freezes is increased. From data reported in these experiments, it would seem that each successive freeze weakens the plants more and more so they are less able to withstand subsequent freezes.

There appeared to be a high degree of association between percent of top growth killed and plant mortality. This was indicated by correlation coefficients of .958 and .883 at 59 and 23 degrees of freedom, respectively. As little as 33.5 percent reduction of top growth resulted in a loss of almost 20.0 percent of the plants.

Plants which were apparently dead may have had from 1 to 3 axillary buds which were still alive. Stems arising from these buds did not grow to any great extent, however, and soon died. The early deaths of stems arising from axillary buds may be explained, in part, to the cutting off of vascular connections between tops and roots due to severe injury of the crown tissue by freezing. After water and nutrients above the crown were exhausted the stems of the axillary buds died.

When winter wheat plants are frozen, a freeze at 4 degrees may account for considerably more damage than a freeze at 10 degrees. Data in these experiments were inconsistent, but seemed to indicate that in subsequent freezes after the initial damaging freeze, a freeze at 10 degrees did about as much damage as a freeze at 4 degrees.

A single freeze at 4 degrees during the "spring" condition resulted in approximately twice the damage to plants as compared to a freeze at the same temperature during the "winter" condition when the plant has lost none of its cold hardiness. From the data in these experiments, there appeared to be no essential difference in amount of top growth injured and plants killed by subsequent freezes at 4 degrees during the "winter" stage compared to the "spring" stage.

There is apparently a relationship between the injury to crown tissue and future top growth after freezing. A limited amount of injury to the crown tissue seemed to have little effect on future growth of the crown. Thus, plants continued to function, even though an appreciable amount of the crown was dead, but probably at a disadvantage. If injury to the crown tissue was so severe that the vascular connections between tops and roots were cut off, the plant would probably die as soon as nutrients and water above the dead crown tissue had been exhausted, even though the amount and condition of top growth may have indicated previously that the plant was recovering from the effects of the freeze.

Thus, death of wheat plants from freezing temperatures may not be due to the freezing of the portion of the plant above ground so much as the cutting off of this top growth from the roots through severe injury or death of the crown tissue. This seems to be a reasonable explanation for the great reduction of numbers of plants in Kansas wheat fields during the winter and early spring from what had appeared to be little or moderate loss in stands.

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A STUDY OF THE EFFECTS OF RECURRENT FREEZES ON WINTER WHEAT PLANTS

by

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Introduction

The Kansas wheat crop is subjected each year to freezes throughout the winter season. In some years damaging freezes have occurred after the wheat plants started their spring growth. The causes of winter killing have been fairly well determined, but very little research has been carried on to determine the effect of sudden low temperatures on plants which have already withstood one or several previous damaging freezes. It would seem an advantage to both the research man and the farmer to have a better understanding of the effects of repeated freezings on the wheat plants. It was with this purpose that these experiments were carried on.

Materials and Methods

Pawnee wheat planted in the fall of 1951 was artificially frozen in a series of experiments at Manhattan, Kansas, beginning December 26, 1951 and ending February 23, 1952. The plants were grown outside in order to attain winter hardiness under as near natural conditions as possible. A thermostatically controlled refrigeration plant was used to provide the cold temperatures. The design of the experiment was such that plants could be frozen for the first, second, third, fourth, and fifth times on a single freezing date; i.e., they were all in the freezing chamber at the same time. A uniform freezing period of 23 hours, beginning at three o'clock was used throughout the experiment. Plants were allowed one week to recover outside the greenhouse before being frozen again.

A 10 pot sample of each freezing group was placed in the greenhouse for observation under conditions more favorable for growth. The relative effect of the recurrent freezes was measured by estimating the percent of top growth apparently killed and determining the percent of plants killed. The percent of top growth injury was estimated at 4, 8, and 12 days after freezing, while the percent of plants killed was determined 8, 16, and 24 days after freezing.

In another part of the experiment crowns of frozen and unfrozen plants were examined and compared. Each crown was cut longitudinally through its center. Care was taken to cut the crown in a manner so as to show a portion of the stem and the root system arising from the basal section of the crown as well as the crown tissue itself. The extent of injury to the interior crown tissue was compared with that of the top growth and with the percent of plants killed in the particular freezing group from which the crown was taken and with the ability of the plant to reestablish itself after the crown had been damaged.

Results

Under laboratory conditions, where no snow cover or other natural protection was present, 3 freezes at a temperature of 4 degrees resulted in injury so severe as to make the wheat plants no longer of economic value. As few as 5 recurrent freezes at this temperature under the above conditions resulted in 100 percent killing of the wheat plants.

Plants not previously frozen artificially seemed to be able to withstand considerable injury to top growth before plants were killed. An increasing proportion of top growth injury and plants killed resulted as the number of successive freezes was increased. One artificial freeze resulted in the death of 19 percent of the plants. Of the plants remaining, 42 percent were killed by a second freeze. A third freeze killed 62 percent of the plants still alive after the second freeze, while a fourth freeze resulted in the death of 88 percent of the plants remaining after the third freeze. At the end of the fifth freeze all top growth and plants were dead. From data reported in these experiments, it would seem that each successive freeze weakens the plants more and more so they are less able to withstand subsequent freezes.

There appeared to be a high degree of association between percent of top growth killed and plant mortality. This was indicated by correlation coefficients of .958 and .883 at 59 and 23 degrees of freedom, respectively.

When winter wheat plants are frozen, a freeze at 4 degrees may account for considerably more damage than at freezes of 10 degrees. Data from experiments comparing the 2 levels of freezing were inconsistent, but seemed to indicate that in subsequent freezes after the initial damaging freeze, a freeze at 10 degrees did about as much damage as a freeze at 4 degrees.

A single freeze at 4 degrees during the "spring" condition when plants had lost a portion of their cold hardiness accounted for much more damage to plants as compared to a freeze at the same temperature during the "winter" condition when the plants had lost none of their cold hardiness.

There is apparently a relationship between the injury to crown tissue and future top growth after freezing. A limited amount of injury to the crown tissue seemed to have little effect on future crown and plant growth. Plants continued to function, even though an appreciable amount of the crown was dead, but probably at a disadvantage. If injury to the crown tissue was so severe that the vascular connections between tops and roots were cut off, the plant would probably die as soon as nutrients and water above the dead crown tissue had been exhausted, even though the amount and condition of top growth may have indicated previously that the plant was recovering from the effects of the freeze.

Plants which were apparently dead may have had from 1 to 3 axillary buds which were still alive. Stems arising from these buds did not grow to any great extent, however, and soon died. The early deaths of stems arising from axillary buds may be attributed, in part, to the cutting off of vascular connections between tops and roots due to severe injury of the crown tissue by freezing. After water and nutrients above the crown were exhausted the stems of the axillary buds died.

Thus, death of wheat plants from freezing temperatures may not be due to the freezing of the portion of the plant above ground so much as the cutting off of this top growth from the roots through severe injury or death of the crown tissue. This seems to be a reasonable explanation for the great reduction in numbers of plants in Kansas wheat fields that sometimes occurs during the winter and early spring.